

FROM THE DEPARTMENT OF UROLOGY (DIRECTOR: PROF. GÖSTA JÖNSSON),
THE UNIVERSITY HOSPITAL, LUND, SWEDEN

Torgny Nilsson

ON SOME HISTOCHEMICAL AND BIOCHEMICAL
ASPECTS OF THE HUMAN PROSTATE

Lund 1973

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Translated by L. James Brown



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The thesis is based on the following papers:

- I. Müntzing, J. & Nilsson, T.: Enzyme activity and distribution in the hyperplastic and cancerous human prostate. Scand. J. Urol. Nephrol. 6, 107-111 (1972).
- II. Nilsson, T., Schueller, E. & Staubitz, W.: β -Glucuronidase activity of the epithelial cells and stroma cells in the hyperplastic prostate. Invest. Urol. 1973. In press.
- III. Nilsson, T. & Müntzing, J.: Histochemical and biochemical enzyme studies in prostatic carcinomatous tissue before and during treatment with estrogen. Scand. J. Urol. Nephrol. 7, 14-17 (1973).
- IV. Nilsson, T. & Müntzing, J.: Histochemical and biochemical investigation of advanced prostatic carcinoma treated with estramustine phosphate, Estracyt[®]. Scand. J. Urol. Nephrol. 7, 13-22 (1973).
- V. Nilsson, T. & Müntzing, J.: Estracyt[®] in advanced prostatic carcinoma. Scand. J. Urol. Nephrol. 6, 11-16 (1972).
- VI. Müntzing, J. & Nilsson, T.: Lipofuscin in malignant and non-malignant human prostatic tissue. Z. Krebsforsch. 77, 166-170 (1972).

In the text these papers are referred to by their Roman numerals.

INTRODUCTION

The human prostate has been the subject of many histochemical investigations. Ever since 1941, when Gomori discovered that the phosphatase activity of this organ is higher than that of any other part of the body, the prostate has been the subject of increasing research. This discovery soon prompted many investigations of the acid phosphatase activity in malignant and non-malignant prostatic tissue, mainly in the hope that it would be useful for checking the histological diagnosis. For it is sometimes a difficult problem histologically to distinguish between well differentiated prostatic carcinoma and non-malignant prostatic cells. The high acid phosphatase activity in the prostate and in metastases of prostatic carcinoma was thought to be a fast and reliable index for identifying the tissue specimens - especially fresh frozen sections (Mathes & Norman, 1956; Rubin, 1969). Most of the histochemical investigations were concerned with acid and alkaline phosphatases (Brandes & Bourne, 1956; Reiner et al., 1957; Butler et al., 1959; Goetsch, 1960; Brandes & Bourne, 1963; Kirchheim et al., 1964; Aso et al., 1968; Feustel et al., 1970; Feustel et al., 1971). Further enzyme systems, such as oxidative enzymes (Brandes & Bourne, 1956; Niemi et al., 1963; Kirchheim et al., 1964; Kirchheim et al., 1966; Prout et al., 1965; Blennerhassett et al., 1967; ElHilali et al., 1968; Belitzky et al., 1970; Feustel et al., 1970; Feustel et al., 1971) esterases (Brandes & Bourne, 1956; Butler et al., 1959; Brandes & Bourne, 1963; Kirchheim et al., 1964; Kirchheim et al., 1966; Niemi et al., 1963), aminopeptidases (Kirchheim et al., 1964; Kirchheim et al., 1966; Niemi et al., 1963) have been studied extensively. As for the localization and distribution, it is generally agreed that most of these enzymes occur within the epithelial cell. However, the amount of histochemically determined enzyme activity in malignant prostatic tissue, in non-malignant prostatic tissue and in different hormonal conditions is still debatable. Several reasons might be offered for the discrepancy in the interpretation of the enzyme activities. The disadvantage of histochemical methods in

estimating activities is well known since the staining intensity depends on various cytological and histological factors. In as much as the enzyme activity is confined to the cytoplasm the visual interpretation is influenced by e.g. cell size and absolute volume of cytoplasmic mass. The thickness of the frozen section also influences the quantitation, as does vacuolization of the cytoplasm (Reiner et al., 1957). The histochemical activity determinations must therefore be regarded as semiquantitative. Despite their limitations, these methods have been widely used. The most obvious controversial point in the opinion of the enzyme activity content is the amount of acid phosphatase and leucine aminopeptidase activity determinations. Several authors claimed that acid phosphatase activity is lower in cancer cells than in normal or hyperplastic cells (Brandes & Bourne, 1956; Goetsch, 1960; Brandes & Bourne, 1963; Kirchheim et al., 1964; Kirchheim et al., 1966), while others are of the opposite opinion (Aso et al., 1968; Feustel et al., 1970; Feustel et al., 1971), others cannot find any differences in acid phosphatase activity between specimens from normal hyperplastic or cancerous specimens (Mathes & Norman, 1956; Reiner et al., 1957; Blennerhassett et al., 1967; Butler et al., 1959). Such differences in opinion clearly indicate the need of methods which not only locate the enzyme, but also give quantitative data.

The biochemically determined enzyme activity has so far been confined to acid phosphatase and leucine aminopeptidase. This might be due to the agreement between histochemically proven activity and the intracellular distribution of the other enzymes studied. To meet the requirements of biochemical activity determinations Woodard & Dean (1947) measured the acid phosphatase activity in the prostatic tissue. They found that the biochemically measured activity was lower in prostatic carcinomatous cells than in normal or hyperplastic tissue (Woodard and Dean, 1947; Woodard, 1956). These results were corroborated by Marberger (1956). Downey et al. (1954) used both histochemical and biochemical methods for measuring acid phosphatase activity. Biochemically, the enzyme activity in homogenates from prostatic carcinomatous tissue was

lower than in tissue specimens of benign prostatic hyperplasia. The determinations were correlated with wet weights (units per gram tissue). However, histochemical examinations showed more intense staining in malignant tissue than in non-malignant tissue indicating a higher enzyme activity in the malignant tissue. The authors could not offer any explanation for the apparent discrepancy and concluded: "Further investigations of phosphatase content of the malignant gland is highly necessary." From their study on zinc and acid phosphatase in the human prostate Hoare et al. (1956) felt that the acid phosphatase is lower in malignant than in non-malignant tissue. Using quantitative histochemistry on tissue specimen from human prostates Parkin et al. (1964) found that the acid phosphatase activity was proportional to the number of acini present. The occurrence of cancer cells in the acini lowered the acid phosphatase activity. They also reported lower activity after stilbestrol therapy. Aso et al. (1972) compared the activity of acid phosphatase in benign prostatic hyperplasia with that of prostatic cancer and specimens from castrated patients with prostatic cancer. These authors, in contrast with others, found an increased amount of acid phosphatase activity in prostatic cancer. The activity was markedly reduced after castration. They did not discuss the difference between their results and those reported by other workers in the field.

According to Niemi et al. (1963), the leucine aminopeptidase activity in benign hyperplasia, as determined biochemically, is only one tenth of that in the normal gland. They did not report anything about activities in the cancerous tissue. These biochemical findings should be compared with the results of Kirchheim et al. (1964), who postulated the possibility of using the missing aminopeptidase activity in prostatic carcinoma (histochemically proven) as a criterion for malignancy.

Among other histochemical findings demonstrating a difference between the malignant and non-malignant prostatic tissue were those on the occurrence of lipofuscin pigment. Lipofuscin pigment

is regarded as waste pigments derived from the hydrolase-rich lysosomes (Brandes, 1966; Köhl, 1968; Ericsson, 1969; de Duve, 1970; de Robertis, 1970). They are derived by the oxidation and polymerization of unsaturated tissue lipids or lipoproteins. The pigments are widely distributed in the body of man, lower animals, fowls, reptils and fishes (Thompson, 1966). Strehler et al. (1959) felt that the lipofuscin pigment was an accumulation of metabolic side products which "figuratively clog up the chemical machinery of the cell". The increased amount of lipofuscin in certain pathological conditions is well known (Strehler et al., 1959; Björkerud & Schelin, 1964; Thompson, 1966). It is apparently generally agreed that lipofuscin pigment is derived from the lysosomes (Daems et al., 1959; Köhl, 1968; Ericsson, 1969). The localization of lipofuscin pigment in the human heart, liver and endocrine organs might often be ascribed to aging, cachexia, poisoning etc. In 1934 Hamperl, who used fluorescence microscopy, reported lipofuscin pigment in the human prostate. In a comparative study between the prostates of young and old individuals, Brandes (1966) found the most characteristic finding to be the marked accumulation of lipofuscin pigment in the epithelial cells of the prostate in the aged, which might be derived from the normal breakdown of phospholipids and proteins "and may represent a progressive deposition in the course of aging of lipid residues resulting from the poor lipolytic activity of the lysosomes".

The aims of the present investigation were

to find biochemical parameters for differentiating between malignant and non-malignant prostatic tissue,

to elucidate the effect of estrogen on prostatic carcinoma with the aid of the above mentioned parameters,

to compare biochemically and histochemically the effect on malignant prostatic tissue of estramustine phosphate and of estrogen treatment, respectively, and

to assess histochemically the amount of lipofuscin pigment derived from the hydrolase-rich lysosomes in malignant and non-malignant prostatic tissue, and to find out whether the values vary with the method of treatment used.

MATERIAL AND METHODS

Prostatic tissue specimens were obtained from inpatients at the Department of Urology at the University Hospital of Lund (38 patients) and at the E.J. Meyer Memorial Hospital, State University of New York at Buffalo (9 patients) during 1970 - 1972. The patients' ages ranged from 49 to 84 years. The two groups did not differ significantly from each other in age distribution. The non-malignant tissue specimens (13) were obtained at transvesical prostatectomies. The malignant tissue specimens were obtained at perineal biopsies a.m. Veenema (Veenema, 1953). Each biopsy specimen was divided into three equal parts: one for histological examination, one for histochemical and one for biochemical studies. After treatment with either estramustine phosphate {estradiol-3-N-[bis-(2-chloroethyl)]-carbamate-17-dihydrogen phosphate; Estracyt®} *) or estrogen (polyestradiol phosphate + ethinylestradiol) for two months, the biopsies were repeated. In this way it was always possible to compare biopsies obtained before and during treatment of each prostate. All together 95 biopsies were performed. None were complicated.

Microscopic examinations

The histological and cytological diagnoses of the malignant prostate specimens were well, moderately and poorly differentiated prostatic carcinoma. Owing to the small numbers in each group it was not possible to demonstrate any correlation between the biochemical results and the degree of differentiation. In the non-malignant group the histological diagnosis was benign prostatic hyperplasia. These investigations were performed at the Departments of Pathology and Cytology at the University Hospital of Lund.

*) See page 25

The histochemical and biochemical methods used were those which had been applied previously in the laboratory in animal experiments. They were known to give reliable results (Müntzing, 1969; Andersson & Müntzing, 1971 A; Andersson & Müntzing, 1971 B; Andersson & Müntzing, 1972; Müntzing, 1972). The methods are described in Andersson & Müntzing (1972) and their modifications in I and VI. Brief summaries of the methods are given below.

Histochemical methods

Immediately after the biopsy had been done, the pieces of tissue used for histochemical studies were immersed in cold neutral 10 % formalin containing 1 % calcium chloride. After 24 hours the specimens were washed in cold distilled water and transferred to a cold 20 % succose solution containing 1 % gum acacia. After a period of 2 - 5 days in this solution the specimens were quenched in liquid propane at -193°C . Cryostat sections (10 μm) were prepared and incubated for demonstrating the enzyme activities.

The methods used (Thompson, 1966) are shown in Table 1 (from Andersson & Müntzing, 1972).

Table 1

Enzyme	Substrate	Coupling Agent	Incubation Time hr
Acid phosphatase	Naphtol-AS-TR-phosphate	Hexazonium para-rosanilin	*)
Alkaline phosphatase	Sodium α -naphtyl phosphate	Fast blue B	0.5
Nonspecific esterases	Naphtol-AS acetate	Fast blue BB	1
Aminopeptidase	L-leucyl- β -naphtylamide	Fast blue B	1
β -Glucuronidase	6-bromo-2-naphtyl- β -D-glucuronide	Fast blue B	6

*) The incubation time for acid phosphatase was 10 min. in benign prostatic hyperplasia and 30 min. in prostatic carcinomatous tissue indicating the higher activity of acid phosphatase in the hyperplastic specimens.

Lipofuscin determinations. The tissue specimens were fixed in cold 10 % formalin, sectioned (10 μ m) with the freezing microtome, mounted in glycerine, and examined in the fluorescence microscope with dark field illumination. With an activating filter with the peak between 390 and 410 μ m and a secondary filter with high absorption below 490 μ m the pigment granules fluoresced golden yellow.

Biochemical methods

The tissue specimens used for biochemical studies were immediately frozen and kept at -70°C until the determinations were performed. The tissue specimens were homogenized in an all-glass tissue grinder. The final concentration was 10 mg per ml. The activi-

ties were determined in the way described by Andersson & Müntzing (1972).

Acid and alkaline phosphatase activities were determined by employing p-nitrophenyl phosphate as substrate (Bessey et al., 1946; Sigma Chem. Bull., 1963). The enzyme activities are expressed in Sigma Units (S.U.). One S.U. is defined as that amount of enzyme which liberates one μ mole p-nitrophenol from p-nitrophenyl in 1 hour.

Non-specific esterase activity was determined by employing a substrate solution containing p-nitrophenyl acetate. One unit of non-specific esterase activity is defined as that amount of enzyme which liberates one μ mole p-nitrophenol from p-nitrophenyl acetate in 1 hour.

Leucine aminopeptidase activity was determined according to Sigma Chemical Co. Bulletin No. 251 (1965) employing α -leucyl- β -naphthylamide as substrate and the activity expressed as Goldbarg and Rutenberg units (G-R units) (Goldbarg & Rutenberg, 1958).

β -Glucuronidase activity was determined with phenolphthalein glucuronic acid as substrate. One unit of β -glucuronidase activity is defined as that amount that liberates one μ g of phenolphthalein from phenolphthalein glucuronic acid in 1 hour (Talalay et al., 1946).

RESULTS

Histochemical results

The acid phosphatase activity was confined mainly to the epithelial cells and sometimes also to the stroma adjacent to the prostatic acini. The products of the reaction for acid phosphatase was seen as a granular precipitate. The amount of acid phosphatase was always lower in the cancerous than in the non-cancerous cells (I). In those malignant cells responding with the usual histological manifestations to estrogen or estramustine phosphate treatment staining intensity was less intense than before therapy (III, IV).

Alkaline phosphatase activity in malignant and non-malignant tissue was found only in the walls of the blood vessels of the stroma (I). The activity was the same in benign and malignant tissue, and neither estrogen nor estramustine phosphate therapy influenced the activity (III, IV).

The site of non-specific esterase activity recognized as granular precipitate was seen in the cytoplasm of epithelial cells. Some activity was located in the stroma. The activity, reflected as staining intensity, was lower in cancerous cells than in non-malignant cells, but the distribution was unchanged (I). No difference in staining intensity was found between specimens taken before and after treatment with estrogen or estramustine phosphate (III, IV).

The leucine aminopeptidase activity was seen as fine granular precipitate in most non-cancerous epithelial cells. But the staining activity varied widely. In some acini of the non-malignant cells no activity could be seen. The distribution of the enzyme activity in malignant cells was the same as in non-

malignant cells, but the intensity was markedly less in the malignant tissue. One tissue specimen from moderately differentiated carcinoma was an exception in that the cancerous cells showed the same staining intensity as non-cancerous cells (I). Neither estrogen nor estramustine phosphate treatment had any appreciable effect on the activity or distribution of the enzyme (III, IV).

The β -glucuronidase activity was distributed only in the cytoplasm of the epithelial cells. There was no difference in distribution or in staining intensity between malignant and non-malignant cells (I). Therapy with estrogen or estramustine phosphate did not affect the localization or degree of activity of the enzymes in cancerous cells (III, IV).

Lipofuscin pigment obtained from the hydrolase-rich lysosomes was visualized with the fluorescence microscopic technique. Benign cells exhibited yellow fluorescent granules of various sizes. The number of granules varied widely from cell to cell. Sometimes clusters of pigment granules were seen in fibromuscular stroma. In non-treated carcinomatous cells these yellow fluorescent granules were less numerous than in benign prostatic hyperplasia. Tissue specimens of lymphoglandular metastases of prostatic origin showed no lipofuscin at all (VI). Even long-term treatment with estrogen produced no increase in the amount of lipofuscin in the cancerous cells. The amount of granules after estramustine phosphate therapy was increased (V, VI).

Biochemical results

The biochemically measured enzyme activities of the five hydrolases, acid and alkaline phosphatases, non-specific esterase, leucine aminopeptidase and β -glucuronidase, showed that the activity of acid phosphatase was reduced in malignant tissue (for

details see I). The leucine aminopeptidase activity in non-malignant tissue specimens varied widely. The activity in malignant tissue was much lower than that in non-malignant tissue, but still measurable. The activity of the other enzymes studied did not differ significantly between cancer and non-cancerous tissue (I).

The β -glucuronidase activity in benign prostatic hyperplasia was registered in separated stroma and epithelium. When good separation was achieved, i.e. when the stroma contained no histologically demonstrable epithelial cells, no β -glucuronidase activity could be found in the stroma (II).

No characteristic difference in enzyme profile could be recognized between malignant and non-malignant tissue.

However, calculations of the quotients of the activities showed that the quotient between acid phosphatase and β -glucuronidase was almost constant in malignant and non-malignant tissue, but differed from each other. The quotients were 2 in malignant and 20 in non-malignant tissue specimens. This was the most obvious and reproducible difference. Other quotients calculated showed no such statistically significant difference. However, the β -glucuronidase/aminopeptidase ratio in cancer was roughly three times that in hyperplasia, and the acid phosphatase/aminopeptidase ratio in hyperplasia was consequently three times that in malignant prostatic tissue (I).

The same ratio as in prostatic carcinoma was found between the acid phosphatase and β -glucuronidase in tissue specimens of lymphoglandular metastases of prostatic origin (I, V).

Prostatic biopsy was performed in two cases originally diagnosed clinically as prostatic carcinoma. The quotient between acid phosphatase and β -glucuronidase differed considerably from that found in benign hyperplastic and in malignant prostatic tissue specimens (I). The histological examination revealed malignant

rectal and bladder changes involving the prostate.

The biochemically determined enzyme activities in prostatic carcinomatous tissue were more or less depressed during estrogen treatment (III). The most conspicuous reduction was that in acid phosphatase activity. The effect of estramustine phosphate treatment (IV) was largely the same as that of conventional estrogen treatment. The quotient between acid phosphatase and β -glucuronidase was less than 1 in nearly all of the patients who had primarily received estrogen or estramustine phosphate therapy. In the untreated patients the quotient was 2 - 3. In those patients who had received estrogen therapy but did not respond to the treatment and who were switched over to estramustine phosphate therapy, the enzyme activities fell still more. The acid phosphatase/ β -glucuronidase quotient was consequently lower when therapy affected the cell.

DISCUSSION

The present investigation compared some histochemical and biochemical variables in the human prostate. The following hydrolases were studied: acid and alkaline phosphatases, non-specific esterase, β -glucuronidase and leucine aminopeptidase. In addition, the amount of lipofuscin was used as a measure of the cytotoxic effect on the cell. The above variables were chosen because the hydrolases, acid phosphatase and leucine aminopeptidase were known to be of significant physiological importance. These enzymes have previously been shown histochemically and unanimity has been achieved on its distribution and localization. But the activity found has varied from one investigation to another. Publications on biochemical determinations of the enzyme activity of the above hydrolases are sparse.

The acid phosphatase and the aminopeptidase are believed to be secretion products of the prostatic epithelial cells. The physiological role of acid phosphatase is presumably to mobilize the energy-rich compounds of adenosindiphosphate (ADP) and adenosin-triphosphate (ATP) and biologically active choline from phosphoryl choline and thus make them available to spermatozoa (Hudson & Butler, 1950; Mann, 1963; Mattila, 1967; Mattila, 1969). The main physiological function of prostatic aminopeptidase is probably to continue the liquefaction of semen started by the other proteolytic enzymes of the prostate (Mann, 1963). The functions of the other hydrolases studied are obscure; β -glucuronidase plays a role in the detoxication of toxic materials and in steroid metabolism (Documenta Geigy, 1962).

In view of the physiological importance of these enzymes and their distribution, it was considered worth while to study them biochemically in non-malignant and malignant tissue specimens for any correlation with the therapeutic results.

In most histochemical and biochemical studies of this type the enzyme activities in different prostate glands are compared with

each other. This might be misleading owing to notable inter-individual differences between the prostates owing to their complex anatomical structure and hence to their varying amount of enzymes. In this study the results were compared with each other in repeated biopsy specimens from the same prostate. Each biopsy specimen was histologically examined and was known to be representative. When biopsy specimens from different parts of the same prostate were studied the activities found were similar. This together with the reproducibility of the results indicate the reliability of the biopsy method, i.e. it produced representative specimens.

The clinical material consisted of consecutive patients. They were grouped according to therapy. The biochemical determinations were performed in the same way in the whole material. The values given in the different papers are therefore comparable.

The histochemical localization of the enzymes studied in the present report has previously been extensively described (Brandes & Bourne, 1956; Mathes & Norman, 1956; Brendler, 1956; Fishman et al., 1959; Niemi et al., 1963; Kirchheim et al., 1964, Kirchheim et al., 1966). The results presented were largely in agreement with those on record. No enzymes were missing completely in the malignant cells. The lack of leucine aminopeptidase activity (histochemically determined) in prostatic cancer cells has been pointed out by Kirchheim as a possible criterion for malignancy. This may, however, be misleading for Niemi and Kirchheim himself have described nodules with no leucine aminopeptidase activity at all in benign prostatic hyperplasia. In the present investigation prostatic cancer cells were found to contain small amounts of histochemically identified aminopeptidase activity, and in one patient the activity was as high as that in benign prostatic hyperplasia.

The biochemical results in this study corroborate the histochemical estimations of the enzyme activities and thereby lend support to the opinion that malignant cells have a measurable amount of

leucine aminopeptidase activity. Judging from these results, then, absence of aminopeptidase activity is not a definite sign of malignant disease of the human prostate. As expected (Woodard & Dean, 1947; Woodard, 1956; Marberger, 1956; among others) the acid phosphatase activity in the malignant cells was lower than that in the hypertrophic cells. It was, however, not possible to recognize any typical biochemical profile of non-malignant or malignant prostatic tissue.

According to Kirchheim et al. (1964, 1966) one of the advantages of quantitative histochemical analysis was that it might be possible to determine the amount of enzyme activity in situ. Most of the enzymes studied are localized mainly to the epithelium. Even specimens from one and the same prostate gland vary in their relative amounts of epithelial and stroma elements. Biochemically determined enzyme activity in homogenates of the biopsies will show the sum of activity of the stroma and epithelium. To overcome this disadvantage without loss of the possibility of quantitative biochemical assay the activities were expressed as quotients.

The β -glucuronidase activity was chosen as a measure of the epithelial cell mass. - Histochemically the β -glucuronidase activity is generally described as being confined to the epithelial cell. This opinion was supported by the determinations of the activity in stroma and epithelium (II) which showed that the stroma when devoid of all epithelium (Franks et al., 1970), contains no measurable β -glucuronidase activity. Furthermore, the acid phosphatase/ β -glucuronidase quotient in the epithelial cells was of the same magnitude as in benign prostatic hyperplasia before separation and thereby excluded the possibility of additional activities from the stroma. Additional activities from the stroma might have been possible if the relative activities had been the same as in the epithelium, but, judging from the above findings, they were not. The quotient in benign prostatic hyperplasia was 10 times that in malignant tissue. In tissue specimens from lymphoglandular metastases of prostatic origin the ratio was the

same as in the primary tumour (I, V). In biopsies of malignant rectal and bladder tissue with involvement of the prostate the ratio between the acid phosphatase and β -glucuronidase activity differed from that found in hyperplasia and cancer, respectively. The value of the ratio thus suggests a biochemical sign for recognizing cancer of prostatic origin (I).

The acid phosphatase/ β -glucuronidase ratio was one or less than one in most cases treated with estrogen. This has been ascribed to a reduction of acid phosphatase activity rather than to a relative increase in the β -glucuronidase activity. This assumption is supported by the histochemical findings indicating a reduced acid phosphatase activity but no appreciable difference in β -glucuronidase activity in untreated and estrogen treated specimens of prostatic carcinoma. It was thus possible biochemically to demonstrate an effect of estrogen on the tumour (III) (Fergusson & Franks, 1953; Franks, 1960). The biochemical effect of primary estramustine phosphate treatment resembles that of estrogen treatment (IV). However, in tissue specimens from those patients previously treated with estrogen but later with estramustine phosphate instead, the enzyme activities and the quotient were lower than before treatment (IV).

Clinical reports (Jönsson & Högberg, 1971; Lindberg, 1972) on advanced cases of prostatic carcinoma have shown that the serum acid phosphatases are reduced after estramustine phosphate therapy, even if they were elevated during previous conventional estrogen therapy. This indicates an effect of estramustine phosphate in addition to that of estrogen.

The results discussed so far do not prove any difference in mode of action between conventional estrogen therapy and estramustine phosphate treatment. However, the estramustine phosphate seems to have a more depressing effect on the enzyme activity in the prostate than conventional estrogen therapy. The fall in serum acid phosphatase after previous estrogen therapy corroborates the above mentioned additional effect.

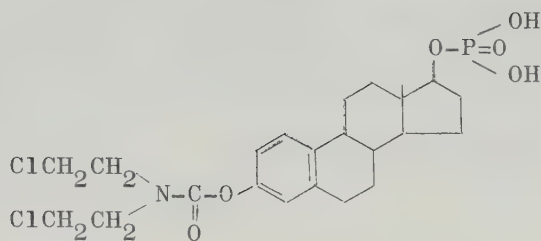
Lipofuscin pigment was studied histochemically for any variation with the therapeutic agents used. The decreased amount of lipofuscin in the cancer cells might be explained by a higher activity of the cancer cells than that of the benign cells. Evidence of an increased amount of lipofuscin in benign prostatic hyperplasia cells has been produced by Hamperl (1934), Brandes (1966), Ablin (1973) and Azzopardi (1972). The markedly increased amount of lipofuscin pigment after estramustine phosphate therapy might suggest a toxic effect on the cancer cells. These findings show a histochemical discrepancy between conventional estrogen therapy and estramustine phosphate in their effect on the cell and suggests that estramustine phosphate has by far the stronger effect on the cancer cells (IV).

SUMMARY

1. Biochemical determinations of the enzyme activity corroborated histochemical measurements of the following enzymes: acid and alkaline phosphatases, non-specific esterase, leucine aminopeptidase and β -glucuronidase. A measurable amount of leucine aminopeptidase activity was found both histo- and biochemically in malignant and non-malignant tissue. The acid phosphatase/ β -glucuronidase ratio in benign prostatic hyperplasia was 10 times as high as that in cancerous tissue. The quotient was the same in prostatic lymphoglandular metastases as in cancerous tissue, but it differed from that in prostatic metastases from rectal and bladder carcinoma.
2. Prostatic cancerous tissue specimens from patients treated with estrogen showed biochemically depressed enzyme activities. The acid phosphatase activity was markedly reduced; the other enzymes studied, only slightly. The quotient between acid phosphatase and β -glucuronidase fell from 2 - 3 to less than 1 when estrogen had affected the tissue.
3. The same biochemical pattern was obtained in tissue biopsies from patients treated with estramustine phosphate. When the response was good the quotient fell to at least 1. Enzyme activity determinations in tissue specimens from patients who had previously been treated with estrogen but who no longer responded was still lower after estramustine phosphate therapy. This might indicate an effect over and above that of estrogen.
4. Lipofuscin pigment obtained from the hydrolase-rich lysosomes was found in small amounts in benign prostatic hyperplasia, but not in untreated cancerous tissue. Neither did prostatic carcinomatous tissue from patients treated with estrogen show any appreciable amount of lipofuscin. The amount of lipofuscin pigment in the malignant tissue

from patients treated with estramustine phosphate was markedly increased. This might indicate a more pronounced effect on the prostatic cancerous cells than what could be achieved by conventional estrogen therapy.

E s t r a c y t[®]



Estradiol-3-N-[bis-(2-chloroethyl)]/-
carbamate-17-dihydrogen phosphate

Theoretically, the purpose of the compound was to enable transport of the agent to hormone-dependent or hormone-responsive tumours and at the same time and, above all, reduce the systemic effect of the cytotoxic moiety. - It has been postulated (Jönsson, 1971) that a cytotoxic effect might be achieved within the prostate by cleavage of the molecule liberating one cytotoxic and one estrogenic moiety. - Determinations in urine and faeces from dog and man after intravenous injection of ³H in the estrogen moiety and ¹⁴C in the alkylating moiety revealed that the estrogen part was excreted faster, indicating a biological degradation of the carbamate ester. Thin layer chromatography of homogenates of benign human hyperplasia incubated with estramustine phosphate indicated a cleavage of the molecule supporting the view that the alkylating moiety in estramustine phosphate might be split off.

The uterotropic activity of the compound is one seventieth to one hundredth that of 17 β -estradiol (in mice). This weak estrogenic effect cannot explain the clinical results (AB Leo, 1973).

The results of numerous clinical trials revealed that 60 % of the patients with advanced prostatic carcinoma no longer responding to conventional estrogen treatment respond favourably to estramustine phosphate. The most obvious improvement is relief of metastatic pain. Regression of clinically and histologically verified metastases has also been reported. The systemic side effects are few (Jönsson, 1969; Alfthan & Rusk, 1969; Jönsson & Högborg, 1971; Anderes et al., 1971; Lindberg, 1972; Nagel & Kölln, 1972; Klein, 1972).

ACKNOWLEDGEMENTS

Sincere thanks are due to:

Professor Gösta Jönsson, M.D., my teacher, for invaluable advice and criticism;

Bertil Högberg, M.D., Director of Research, for generous support and most valuable criticism as well as for placing the resources of the Research Laboratories, AB Leo, at my disposal;

Jonas Müntzing, Ph.D., for his friendly support, most valuable discussions and criticism;

Members of the staffs of the Departments of Urology, General Surgery, Pathology, Cytology and Embryology for help and friendly discussions; and

Mrs. Gunnel Spégel for skilful assistance in typing the papers.

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